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LIPIDS OF SARCINA LUTEA:

III. COMPOSITION OF THE COMPLEX LIPIDS

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LIPIDS OF *SARCINA LUTEA*: III. COMPOSITION OF THE COMPLEX LIPIDS

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ABSTRACT

The complex lipids of a strain of *Sarcina lutea* were isolated and separated into fractions on DEAE cellulose acetate and silicic acid columns. These fractions were monitored in several thin-layer chromatography systems. The various lipid types were characterized by their behavior in thin-layer systems and by an analysis of their hydrolysis products. The fatty acid composition of the column fractions was determined by gas-liquid chromatography.

Thirteen components were separated by thin-layer chromatography and characterized. The major components were polyglycerol phosphatide (17.0%), lipoamino acids (15.1%), phosphatidyl glycerol (13.8%), and an incompletely characterized substance (15.0%). Minor constituents included phosphatidyl inositol (5.5%), phosphatidic acid (4.2%), phosphatidyl serine (2.0%), and phosphatidyl choline (1.0%). No phosphatidyl ethanolamine was observed.

I. INTRODUCTION

Few bacterial phospholipids have been studied thoroughly by modern analytical techniques. Lovern[1] has described many bacterial phospholipids as simple fatty acid esters of phosphorylated carbohydrates. Ethanolamine is the most commonly reported nitrogen-containing component; chloline is found in some species.[2] Recent reports have also described the isolation of lipoamino acid complexes in the complex lipids of bacteria.[3-6]

We have reported[7] that Sarcina lutea contains a complex mixture of highly polar lipids that comprise approximately 23% of the total extractable lipid. The determination of the composition of this polar fraction is reported in this manuscript.

II. MATERIALS

The preciseness of the experimental procedures and the small quantities of lipids under investigation required extreme purity and reliability in the reagents employed. For these reasons the following materials were obtained from the sources shown:

1) Whatman diethylaminoethyl (DEAE) cellulose — Scientifica, Clifton, New Jersey.

2) Silicic acid, analytical reagent grade — Mallinckrodt Chemical Works, New York, New York.

3) Inorganic reagents, solvents, and Hyflo Super Cel — Fisher Scientific Company, Silver Spring, Maryland.

4) Synthetic phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl choline (all dipalmitoyl) A-grade — Calbiochem, Los Angeles, California.

5) All lipid standards except lecithin and cephalin standards — Mann Research Laboratories, New York, New York; or Applied Science Laboratories, Inc., State College, Pennsylvania.

6) Rhodamine 6G and ninhydrin — Eastman Organic Chemicals, Rochester, New York.

7) Thin-layer chromatography apparatus and materials — Brinkman Instruments, Inc., Great Neck, New York.

Whatman diethylaminoethyl (DEAE) cellulose was converted to the acetate form according to the method of Rouser et al.[8] The silicic acid was sieved to 100/200, 100/140, and 160/200 mesh. Chloroform, hexane, and propionic acid were redistilled before use. The methanol, acetone, benzene, and diisobutyl ketone were gas chromatographically pure. Lecithin and cephalin standards were isolated from soy phosphatides by silicic acid column chromatography according to the method of Fleischer et al.[9] and examined for homogeneity by thin-layer chromatography by the method of Horrocks.[10]

III. EXPERIMENTAL PROCEDURES

A. CULTURE CONDITIONS

Sarcina lutea (ATCC 533) was cultured at 25 C for 24 hours in tryptic soy broth (Difco) under forced aeration. The prepared medium contained less than 0.009% lipid.

Cells were harvested as previously described[7] immediately following the growth period; all operations were performed below 5 C.

B. LIPID EXTRACTION

The method used to extract the lipids and to isolate the complex lipid fraction was described previously.[7] Nonlipid contaminants that were not removed by the Folch washing procedure were separated on Sephadex[11] and on DEAE cellulose[8] columns. These contaminants amounted to 8 to 14% of the complex lipid fraction from silicic acid chromatography and consisted of glycerol, nitrogenous bases, and peptides.

C. COLUMN CHROMATOGRAPHY

Four classes of lipids were isolated on DEAE cellulose acetate columns by the method of Rouser et al.[8] Zwitterionic and neutral lipids were eluted with chloroform - methanol, 7:1 (v/v), basic lipids with chloroform - methanol, 7:3 (v/v), acidic lipids with glacial acetic acid, and highly acidic lipids with chloroform - methanol - concentrated ammonium hydroxide, 80:20:1 (v/v/v). The fraction eluted with chloroform - methanol, 7:1 (v/v), was resolved into neutral lipids, lecithins, and lysolecithins on a silicic acid - silicate column.[8] Free fatty acids were removed from the acidic lipid fraction on silicic acid.[9]

Phosphatidic acid and polyglycerol phosphatide (cardiolipin type) were isolated by elution from an acid-washed silicic acid column with 5% methanol in chloroform. Phosphatidic acid traveled with the solvent front; the polyglycerol phosphatide eluted much later. After rechromatography, each of the fractions gave a single spot on thin-layer plates. None of the other components could be isolated in pure form by column chromatography.

D. THIN-LAYER CHROMATOGRAPHY

Silica gel G plates prepared according to Stahl, or silica gel G plates impregnated with 10% sodium acetate,[10] were developed in the ascending manner in unlined tanks to resolve the various complex lipids and to monitor column fractions. Chloroform - methanol - water, 65:25:4 (v/v/v),[10] chloroform - methanol - 14% aqueous ammonia, 17:7:1 (v/v/v), and diisobutyl ketone - acetic acid - water, 8:5:1 (v/v/v)[12] were used as solvent systems. The spots were detected with (i) aqueous rhodamine 6G, (ii) iodine vapors, (iii) a molybdate reagent,[13] (iv) Dragendorff's reagent for choline and dimethylaminoethyl compounds, (v) 0.25% ninhydrin in acetone buffered to pH 5.5 with acetate, (vi) iodine-sodium azide reagent for sulfolipids,[14] and ammoniacal silver nitrate for phosphatidic acid, phosphatidyl glycerol, polyglycerol phosphatide, and inositides.

In some cases the silicic acid impregnated filter paper system of Marinetti[15] was used to aid in the identification of lipids for which no standards were available.

The material resolved by thin-layer chromatography (TLC) was quantitated according to Amenta.[16]

E. HYDROLYSIS AND PAPER CHROMATOGRAPHY

Water-soluble products, produced by hydrolysis of the various fractions and individual components, were analyzed according to the method of Ikawa,[8] by ascending chromatography in n-butanol - acetic acid - water, 2:1:1 (v/v/v),[17] or in phenol - n-butanol - formic acid - water, 50:50:3:10 (v/v/v/v);[18] and by descending chromatography in n-butanol - acetic acid - water, 4:1:5 (v/v/v),[19] or n-butanol - propionic acid - water according to the method of Benson and Maruo.[20] Phosphate esters were detected with Haynes-Isherwood reagent, amino compounds with 0.4% ninhydrin in water-saturated n-butanol, carbohydrates with 2.5% aniline hydrogen phthalate, polyhydroxy compounds with ammoniacal silver nitrate, and quaternary ammonium salts with Dragendorff's reagent. Whatman No. 1 paper was used for ascending chromatography and Whatman No. 4 paper for descending chromatography.

F. ACETONATION OF VICINAL HYDROXYL GROUPS

Samples suspected of containing either phosphatidyl glycerol or polyglycerol phosphatide were acetonated by the procedure of Benson and Maruo.[20] Samples before and after acetonation were analyzed by TLC on silica gel G in chloroform - methanol - water, 65:25:4 (v/v/v). Detection was as described in Section III, E above.

G. CHEMICAL METHODS

Inorganic phosphorus was determined by the Fiske-Subbarow procedure,[21] total phosphorus by the Allen modification,[22] amino nitrogen according to Lea and Rhodes,[23] and total nitrogen according to Miller and Miller.[24] Acetals and aldehydes were qualitatively sought, using azobenzenephenylhydrazine sulfonic acid.[25] Fatty acids were determined by saponification, methanolysis, and weighing. Fatty acid methyl esters were produced for gas chromatography as described in a previous paper.[7]

H. GAS-LIQUID CHROMATOGRAPHY

Gas-liquid chromatography (GLC) of fatty acid methyl esters was carried out as outlined previously[7] after separation of the normal fatty acid and hydroxy-acid esters on silicic acid columns.[26]

Carbohydrates were separated as their trimethylsilyl (TMS) derivatives[27] on a 4-ft by 1/4-inch column of 3% SE-52 on 60/80 mesh Chromosorb W at various temperatures.

I. INFRARED ANALYSIS

Infrared spectra of various fractions and constituents were examined as thin films on KBr or AgCl plates using a Perkin-Elmer Model 21 recording infrared spectrophotometer.*

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* Perkin-Elmer Corp. Norwalk, Connecticut.

IV. RESULTS

Thirteen components were observed in the complex lipid fraction, as indicated on the TLC tracing shown in Figure 1. The relative amounts of each component and tentative identifications are summarized in Table I. Table II provides data on the weight per cent of the complex lipid, phosphorus, nitrogen, fatty acid, and amino nitrogen content of the various column fractions.

Samples of the total complex lipid material were tested before and after acid hydrolysis for aldehyde, acetal, or sulfhydryl functions. All such tests were negative.

Chromatography of the various fractions on silicic acid-impregnated paper confirmed several of the identifications in Table I, but because of the wide variety of lipoamino acid complexes all of the components could not be resolved by this technique.

TABLE I. COMPLEX LIPID CONTENT OF S. LUTEA

Spot[a]	DEAE Column Fraction[b]	Per Cent of Total Complex Lipid	Tentative Identification
A	N-Z	3.0	Neutral lipid
B	N-Z	1.0	Oxidized glyceride
C	N-Z	8.4	Lipoamino-acid
D	N-Z	6.7	Lipoamino-acid
E	HA	4.2	Phosphatidic acid[c]
F	N-Z,B	8.0	Free fatty acid (salt)
G	B,HA	17.0	Polyglycerol phosphatide[c]
H	B,A	15.0	Unidentified
I	A,HA	13.8	Phosphatidyl glycerol[c]
J	N-Z	1.0	Lecithin
K	HA	5.5	Phosphatidyl inositol[c]
L	A	2.0	Phosphatidyl serine[c]
M	N-Z,B,A,HA	14.4	Nonlipid

a. See Figure 1.
b. N-Z = neutral and zwitterionic, B = basic, A = acidic, HA = highly acidic.
c. Additional confirmation of identity provided by chromatography according to Marinetti.[16]

TABLE II. ANALYTICAL RESULTS OF COLUMN FRACTIONS[a]/

DEAE Column Fraction[b]/	Per Cent of Complex Lipids	Per Cent of P	Molar Ratios[c]/		
			FA/P	Total-N/P	Amino-N/P
N-Z	23.0	0.78	7.56	2.20	2.00
B	17.4	0.54	1.98	2.00	1.80
A	18.8	2.22	1.97	0.12	0.12
HA	26.4	3.56	1.97	2.18	1.94
Phosphatidic acid	4.2	4.87	1.98	0.10	0
Polyglycerol phosphatide	17.0	4.49	1.99	0.12	0

a. Most fractions contained significant amounts of nitrogenous nonlipid, resulting in many of the high N/P ratios.
b. See footnote b, Table I.
c. FA = fatty acid, P = phosphorus, N = nitrogen.

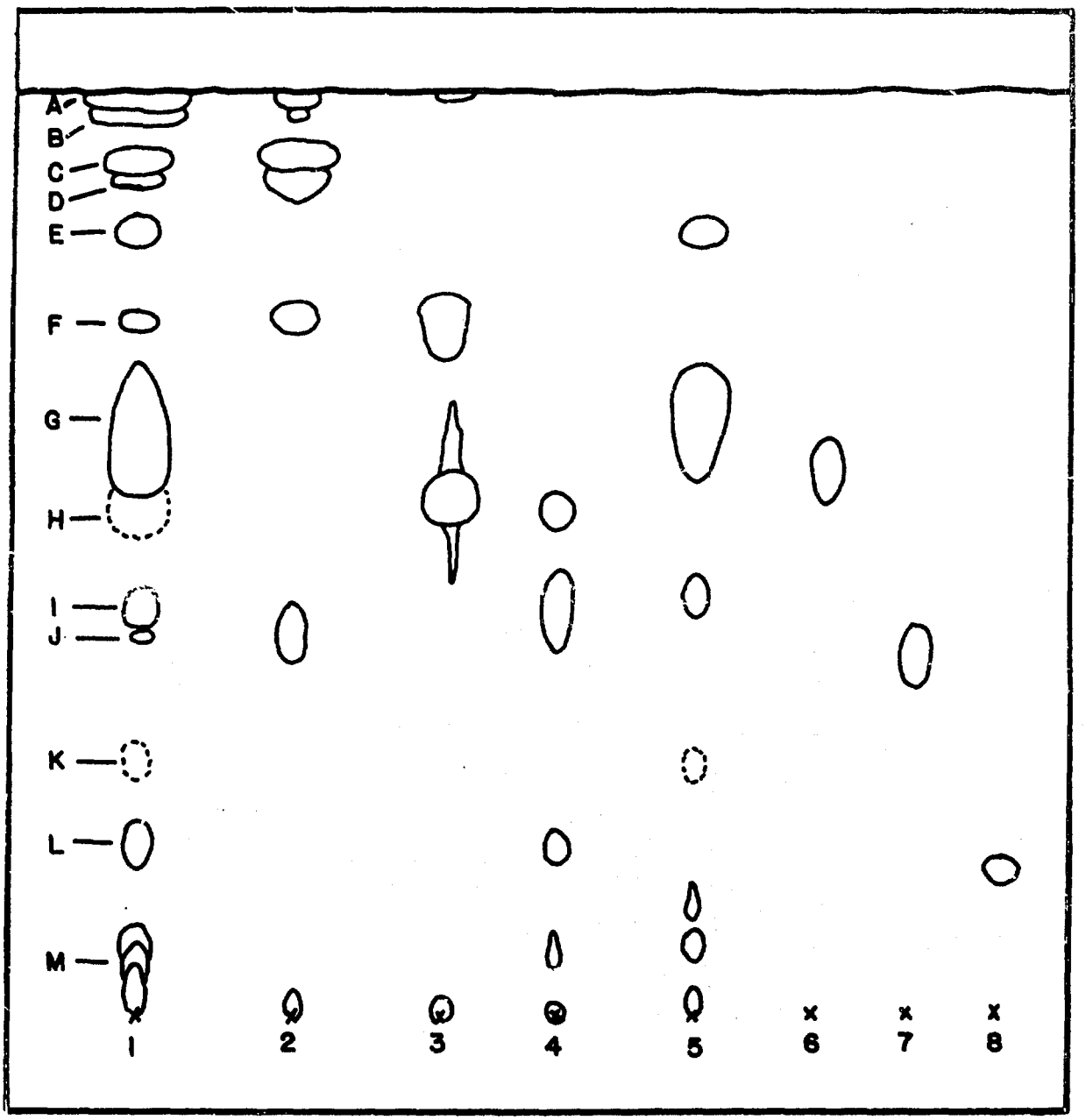

Figure 1. Thin-Layer Chromatography of S. lutea Complex Lipids. Solvent system: chloroform-methanol-14% aqueous ammonia, 17:7:1 (v/v/v). Spots: (1) total complex lipid, (2) neutral-zwitterionic fraction, (3) basic fraction, (4) acidic fraction, (5) highly acidic fraction, (6) authentic phosphatidyl ethanolamine, (7) soy lecithin, and (8) authentic phosphatidyl serine. A detailed discussion of the spots follows on the next two pages.

IDENTIFICATION OF SPOTS IN FIGURE 1, TOTAL COMPLEX LIPID

Spot A - Neutral Lipid. This spot occurred in the neutral-zwitterionic lipid fraction and was eluted from silicic acid with the least polar solvent used. It moved with the solvent front in all TLC systems, gave a yellow color with rhodamine 6G, and did not react with the other detection reagents. On this basis the material was identified as neutral lipid carryover, and was primarily responsible for the high FA/P ratio observed in the neutral-zwitterionic lipid fraction.

Spot B - Oxidized Glyceride. This brown material was easily visible on the TLC plate without treatment. Because it was absent in preparations handled entirely under nitrogen, it was probably oxidized glyceride and not a pigment.

Spot C - Lipoamino acid. This spot was ninhydrin-positive, yellow with rhodamine 6G and, upon hydrolysis, yielded glycerophosphate, phenylalanine, and a material that moved faster than any of the natural quaternary ammonium bases but that reacted with Dragendorff's reagent. The spot was thus considered to be lipoamino acid, the exact structure of which has not been determined. However, since it was of relatively low polarity and nonacidic, we may suppose that the linkage was through the carboxyl group of the amino acid.

Spot D - Lipoamino acid. Serine, threonine, and a trace of phenylalanine were found in the hydrolyzate of this material. The presence of the two hydroxy constituents, serine and threonine, in view of the failure of this material to reduce ammoniacal silver nitrate, suggests the possibility that the ester linkage occurs through the OH-group of the amino acids. Because this material moved much faster than ordinary phosphatidyl serine, a structure other than the usual acylated glycerol phosphate skeleton is postulated. And, since this spot was ninhydrin-positive, the linkage was probably not through the NH_2 group. The amino acid-containing lipids of S. lutea gave indications of great diversity in structure, but complete characterization of each type was beyond the scope of this study.

Spot E - Phosphatidic Acid. This material was highly acidic, easily eluted from silicic acid with 5% methanol in chloroform, and contained no nitrogen. It had a FA/P molar ratio of 1.98 and a phosphorus content of 4.87%, which corresponds almost exactly with the calculated value for diisopentadecanoyl phosphatidic acid. It gave a weak reaction with the molybdate reagent and a strong reaction with a modified Hayes-Isherwood reagent, thus reflecting its oxidizing nature. Only glycerophosphate and fatty acids were found in the hydrolysis products. These data plus the observed Rf's in various TLC systems identified this spot as phosphatidic acid.

Spot F - Salt of Free Fatty Acid. The fatty acid nature of this material was ascertained by its negative reaction with the two molybdate sprays and with ninhydrin when developed in the acid or neutral TLC systems, and by its extraction from the complex lipid mixture with aqueous 0.1 M borax. When developed in the ammoniacal solvent system, this spot held sufficient ammonia to give a transient ninhydrin reaction. Qualitative analysis of this material[28] indicated the absence of calcium, magnesium, and ammonium salts. Sodium ions were found, but could easily be contaminants. The relative size of the spot was larger in older preparations, indicating its formation by slow autohydrolysis of more complex lipid compounds.

Spot G - Polyglycerol Phosphatide. On the basis of TLC data and column chromatographic properties, as well as its reaction with various of the detection reagents, spot G could have been either a bis-phosphatidic acid salt or a cardiolipin type of polyglycerol phosphatide. Hydrolysis of this material, after isolation on silicic acid, gave diphosphoglycerol (Rf 0.13 in butanol - acetic acid - water, 2:1:1, v/v/v), and diglycerol phosphate (Rf 0.17) in the descending system of Benson and Maruo.[20] The spot had a phosphorus content of 4.49%, which would be expected for tetraisopentadecanoyl cardiolipin of the structure described by MacFarlane and Gray.[29] The resulting FA/P molar ratio of 1.99 indicates that the structure is probably of the cardiolipin type rather than a bis-phosphatidic acid, which would have a FA/P ratio of 4:1. Further evidence for the cardiolipin structure was provided by the nearly negligible nitrogen content, failure of the material to reduce ammoniacal silver nitrate (bis-phosphatidic acid reacts), and the extreme tailing on silicic acid columns.

Spot H - Unidentified. This material upon hydrolysis produced a wide variety of constituents including glycerophosphate, serine, the methyl ester of alanine, methyl ethanolamine, and an as yet unidentified fast-moving Dragendorff-positive substance. The appearance of this material primarily in the basic lipid fraction suggests that the nitrogenous moieties are linked through their carboxyl functions. It has, however, not been structurally characterized because it has been impossible to isolate it in a pure form.

Spot I - Phosphatidyl Glycerol. This material was identified by its acidic character, Rf values in the various TLC systems (especially the two-dimensional system of Lepage[28]), loss of ability to react with alkaline silver nitrate after acetonation (see Figure 2), and its response to the various detection reagents. It strongly resisted hydrolysis, probably because of the stability of the glycerol-phosphate bond.[30] Hydroxyl absorption at 2.87 and at 9.43 microns was very prominant in the infrared spectrum of this material.

Spot J - Lecithin. Spot J was identified as lecithin by its nonretention on DEAE cellulose acetate, Rf values in the TLC systems, reaction with Dragendorff's reagent, and the production of glycerophosphate and what appears to be methyl choline upon hydrolysis.

Spot K - Phosphatidyl Inositol. Although this material is reported as comprising 5.5% of the complex lipid (Table I), this value must be taken as a maximum one. GLC of the TMS derivative of inositol released by a 48-hour hydrolysis of this material in 6 N aqueous HCl at 110 C indicated a phosphatidyl inositol content of less than one per cent. However, complete hydrolysis to free inositol is seldom achieved,[31] and the higher value may be more nearly correct. Identification was based on the material's acidic nature, Rf values in various TLC systems, ability to reduce ammoniacal silver nitrate, and recovery of inositol from hydrolyzates. The entire fraction in which this material appeared had a resulting FA/P ratio of 1.98, and the Rf values were fairly high. It is therefore probable that this material was a monophospho-, monoinositol-structure.

Spot L - Phosphatidyl Serine. The concentration of this material varied widely between batches of phospholipid material. It behaved as authentic phosphatidyl serine in various TLC systems, in its reactions with the detection reagents, and in producing serine upon hydrolysis. The reason for its variable quantitation is unknown.

Spot M - Water-Soluble Nonlipid. Much, but not all, of this material was removed from the complex lipid mixture in the methanol fraction from DEAE cellulose acetate. Paper chromatography of this material prior to hydrolysis revealed the presence of choline, serine, phenylalanine, alanine, glycerol, and inorganic phosphate. No fatty acids were recovered from acid hydrolyzates of this material.

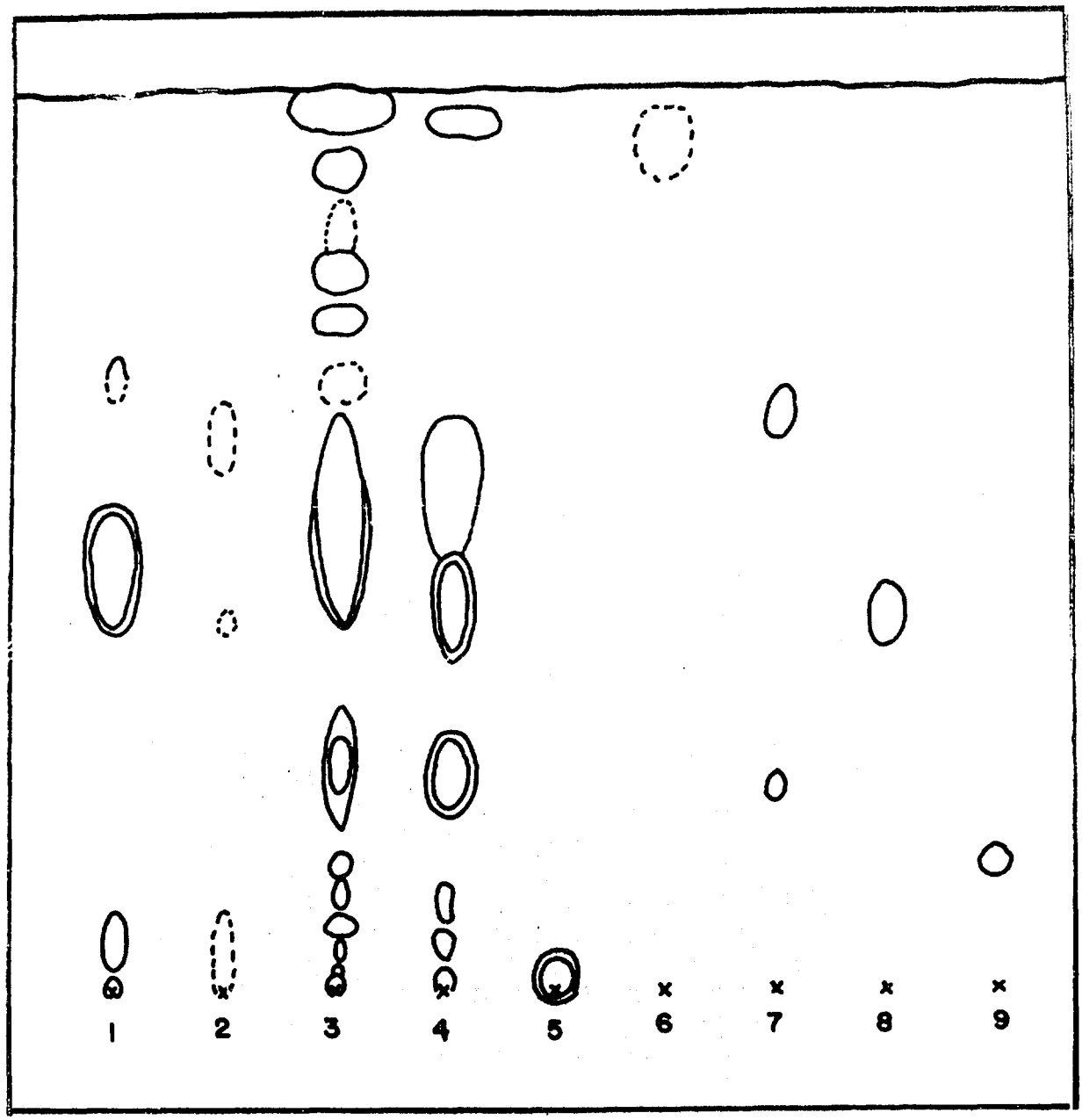

Figure 2. Thin-Layer Chromatography of Phosphatidyl Glycerol and Phosphatidyl Inositol. Solvent system: chloroform-methanol-water, 65:25:4 (v/v/v). Spots: (1) phosphatidyl glycerol fraction from silicic acid column, (2) material from Spot one after acetonation, (3) total complex lipids, (4) highly acidic fraction, (5) inositol, (6) oleic acid, (7) authentic phosphatidyl ethanolamine, (8) soy lecithin, and (9) authentic phosphatidyl serine. Solid lines outline spots detected with molybdate reagent; dotted lines outline spots visible only after exposure to iodine vapors; double lines outline spots detected with alkaline silver nitrate. Spots (1) and (2) are the same concentration.

A. THE LIPOAMINO ACID COMPONENTS

Twenty-four-hour cultures of S. lutea were quite rich in lipids containing amino acid. Alanine, phenylalanine, serine, proline, leucine, isoleucine, valine, arginine, methylalanine, tyrosine, and threonine were detected in various batches of the complex lipids. Some of these may be nonlipid contaminants, but there was evidence that serine, alanine, phenylalanine, leucine, tyrosine, and threonine were structural components of the lipid constituents examined. Phosphoryl-serine, -choline, and -threonine have been observed on paper chromatograms of various hydrolyzates. No positive biuret reaction was observed with any of the preparations.

Cells allowed to stand at 4 C for weeks to months yielded a complex lipid fraction essentially free of amino nitrogen and extremely low in total nitrogen. In these samples almost all of the nitrogenous material was removed by the Folch washing procedure. The only complex lipids found in significant amounts in these preparations were phosphatidic acid, polyglycerol phosphatide, and phosphatidyl glycerol.

B. FATTY ACID COMPOSITION

The fatty acid composition of the various complex lipid fractions is summarized in Table III.

C. SPECTRAL DATA

The infrared spectra of the various complex lipid fractions differed substantially in only two regions. The basic lipids absorbed in the 6- to 6.8—micron region characteristic of the NH group of amino acids,[32] but the acidic lipids did not. The acidic lipids, on the other hand, absorbed conspicuously at 8.25 microns an absorption peak characteristic of secondary amides,[32] but the basic lipids did not.

TABLE III. FATTY ACID COMPOSITION OF S. LUTEA COMPLEX LIPIDS[a]

Fatty Acid Carbon Chain[b]	Total Complex Lipid	Composition, per cent Column Fractions[c] N-Z	B	A	HA	Phosphatidic Acid	Polyglycerol Phosphatide
12	1.0	1.9	0.6	0.2	1.1	4.8	-
12:0 + 12:1	0.3	0.6	0.4	0.3	trace[d]	3.4	trace
br-13:0 + 13:1	0.3	0.5	0.3	0.3	trace	2.6	2.7
br-14:0	1.3	0.6	0.4	0.8	2.9	1.3	0.6
n-14:0	1.7	2.3	0.6	0.6	2.6	4.4	1.0
n-14:1	1.3	3.1	0.7	0.6	0.6	1.3	trace
br-15:0 (iso)	18.4	11.9	23.5	22.4	17.7	3.3	20.3
br-15:0 (anteiso)	41.7	28.1	53.5	52.3	38.3	16.6	43.7
n-15:0	2.1	2.5	0.6	1.7	3.0	trace	1.6
br-16:0	1.6	1.1	0.6	1.4	2.7	1.8	1.5
n-16:0	5.1	11.6	4.5	1.8	2.1	23.5	3.4
n-16:1	7.3	7.7	2.8	3.6	12.5	4.4	6.2
br-17:0	1.5	1.0	0.6	2.0	2.0	trace	2.1
Unidentified	0.4	-	0.2	-	0.3	3.8	-
n-17:0	0.5	1.0	0.4	-	0.4	2.8	0.6
n-17:1	0.2	0.3	0.1	0.8	0.4	0.2	0.9
br-18:0	0.7	2.2	trace	0.2	0.2	-	0.2
n-18:0	11.2	18.8	8.5	6.3	9.8	16.1	5.7
n-18:1 (Δ^{11})	2.8	4.2	1.0	2.0	3.3	8.3	2.5
br-19:0 (iso)	0.5	1.2	trace	0.7	trace	-	3.0
n-19:0	trace	-	0.2	-	-	1.1	trace
br-20:0 (anteiso)	trace	-	trace	-	trace	-	0.9
n-20:0	trace	-	trace	-	-	-	2.7
br-22:0 (anteiso)	trace	-	trace	-	-	1.5	-

a. Quantitation from chromatograms made using an 8-ft by ¼-inch copper column containing 10% butanediol succinate on 80/100 mesh Chromosorb W. Column maintained at 190 C.

b. Number of carbon atoms in acid: number of double bonds; br = branch.

c. See footnote b, Table I.

d. Trace = less than 0.1%.

V. DISCUSSION

The complex lipids of S. lutea as shown in Table I contain fairly large quantities of lipoamino acids, phosphatidyl glycerol, and polyglycerol phosphatide. Lecithin, phosphatidyl serine, and phosphatidyl inositol, common among plant and animal phospholipids, were present in only small amounts; phosphatidyl ethanolamine was not detected at all. Akashi and Saito[33] have previously reported that Sarcina phospholipids consisted principally of phosphatidic acid. Phosphatidyl ethanolamine has been reported as the major phospholipid of Serratia marcescens,[34] Escherichia coli,[35] Azotobacter agilis, and Agrobacterium tumefaciens.[36] The phospholipids of some lactic acid bacteria, however, have been described by Ikawa[6] as containing no serine, ethanolamine, or choline. Instead, the principal ninhydrin-positive lipids of these organisms yielded lysine and alanine upon hydrolysis.

Lipoamino acid complexes amounted to 15.1% of the total complex lipids from S. lutea. These complexes are very labile to enzymatic degradation and may be lost from cells unless enzymatic processes are stopped immediately after the cells are harvested.[3] Gaby, Wolin, and Zajac[37] and Hunter and James[5] have suggested that these complexes are involved in amono acid transport processes. The wide range of amino acids found to be associated with these lipids in the present study seem to confirm this view. However, as has been suggested, these complexes in S. lutea apparently contain both carboxyl and hydroxyl linkages, and possibly (Spot H) even amino linkages. Moreover, the amino acids appeared to be bound to other moieties in addition to phosphatidyl glycerol.

Hunter and Godson[38] have suggested that some lipoamino acid complexes may be transient intermediates in the transfer of amino acid residues from a nucleic acid template in the formation of lipoproteins. Serine, threonine, hydroxylysine, tyrosine, and other amino acids possessing a hydroxyl function may conceivably interchange during or after formation of the classical acidic cephalins. This could account for the variable content of phosphatidyl serine found in different batches of the S. lutea complex lipids.

The possibility exists, then, that the different lipoamino acid types found in S. lutea may serve different functions, some primarily structural components, some involved in amino acid transport, and some intermediates in lipoprotein synthesis.

The fatty acid composition of the total complex lipid (Table III) differs in several respects from that reported previously.[7] The 24-hour culture used in this study was selected to maximize the yield of lipoamino acid complexes.

The fatty acid composition of the phosphatidic acid fraction differed appreciably from that of all the other fractions. If we suppose phosphatidic acid to be an intermediate in the synthesis of other phospholipids,[31] or a product of the enzymatic removal of a nitrogenous base from glycerophosphatides,[39] then this difference is difficult to explain. Of the various fractions shown in Table III, only the basic lipid and the acidic lipid fractions closely resemble each other in fatty acid composition. They also constitute similar percentages of the total complex lipid (17.4 and 18.8%, respectively). It is not unlikely, therefore, that these materials are formed from similar starting materials, e.g., diglycerides, and differ primarily only in the nature and linkage of the nitrogenous constituent.

The polyglycerol phosphatide of S. lutea appears to be a cardiolipin. This type has been reported to be present in Staphylococcus aureus;[4] the presence of the bis-phosphatidic acid-type has been reported in Bacillus polymyxa.[40]

Further discussion on the possible metabolic roles of the various complex lipids of S. lutea will have to await (i) studies on the distribution of these components within the cell, and (ii) characterization of the individual metabolic pathways for each component.

LITERATURE CITED

1. Lovern, J.A. 1957. The phosphatides and glycolipids, p. 376-392. In W. Ruhland (ed.) Handbuch der pflanzenphysiologie. Vol. VII, Springer-Verlag, Berlin.

2. Asselineau, J., and E. Lederer. 1960. Chemistry and metabolism of bacterial lipides, pp. 337-406. In K. Bloch (ed.) Lipide metabolism. John Wiley & Sons, Inc., New York.

3. MacFarlane, M.G. 1962. Characterization of lipoamino-acids as O-amino-acid esters of phosphatidyl glycerol. Nature 196:136-138.

4. MacFarlane, M.G. 1962. Lipid components of Staphylococcus aureus and Salmonella typhimurium, Biochem. J. 82:40P-41P.

5. Hunter, G.D., and A.T. James 1963. Lipoamino-acids from Bacillus megaterium. Nature 198:789.

6. Ikawa, M. 1963. Nature of the lipids of some lactic acid bacteria. J. Bacteriol. 85:772-781.

7. Huston, C.K., and P.W. Albro. Lipids of Sarcina lutea: I. Fatty acid composition of the extractable lipids. J. Bacteriol. 88:425-432.

8. Rouser, G. A.J. Bauman, G. Kritchevsky, G. Heller and J.S. O'Brien. 1961. Quantitative chromatographic fractionation of complex lipid mixtures. J. Am. Oil Chemists' Soc. 38:1-11.

9. Fleischer, S., G. Brierley, H. Klouwen, and D.B. Slautterback. 1962. Studies of the electron transfer system: XLVII. The role of phospholipids in electron transfer. J. Biol. Chem. 237:3264-3272.

10. Horrocks, L.A. 1963. Thin-layer chromatography of brain phospholipids. J. Am. Oil Chemists' Soc. 40:235-236.

11. Wells, M.A., and J.C. Dittmer. 1963. The use of Sephadex for the removal of nonlipid contaminants from lipid extracts. Biochemistry 2:1259-1263.

12. Lepage, M. 1964. The separation and identification of plant phospholipids and glycolipids by two-dimensional thin-layer chromatography. J. Chromatog. 13:99-103.

13. Dittmer, J.C., and R.L. Lester. 1964. A simple, specific spray for the detection of phospholipids on thin-layer chromatograms. J. Lipid Res. 5:126-127.

14. Block, R.J., E.L. Durrum, and G. Zweig. 1958. A manual of paper chromatography and paper electrophoresis. 2nd ed., p. 131. Academic Press, New York.

15. Marinetti, G.V. 1962. Chromatographic separation, identification, and analysis of phosphatides. J. Lipid Res. 3:1-20.

16. Amenta, J.S. 1964. A rapid chemical method for quantification of lipids separated by thin-layer chromatography. J. Lipid Res. 5:270-272.

17. Fink, K., R. Cline, and R. Fink. 1963. Paper chromatography of several classes of compounds. Correlated Rf values in a variety of solvent systems. Anal. Chem. 35:389-398.

18. Goldfine, H. The characterization and biosynthesis of an N-methylethanolamine phospholipid from Clostridium butyricum. Biochim. Biophys. Acta 59:504-506.

19. Block, R.J., R. LeStrange, and G. Zweig. 1952. Paper chromatography. Academic Press, Inc., New York.

20. Benson, A.A., and B. Maruo. 1958. Plant phospholipids: I. Identification of the phosphatidyl glycerols. Biochim. Biophys. Acta 27:189-195.

21. Fiske, C.H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

22. Allen, R.J.L. 1940. The estimation of phosphorus. Biochem. J. 34:858-865.

23. Lea, C.H., and D.N. Rhodes. 1955. The ninhydrin reaction of unhydrolyzed phospholipids. Biochim. Biophys. Acta 17:416-423.

24. Miller, G.L., and E.E. Miller. 1948. Determination of nitrogen in biological materials. Anal. Chem. 20:481-488.

25. Feigl, F. 1954. Spot tests: II. Organic applications, p. 152. Elsevier Publishing Co., New York.

26. Kishimoto, Y., and N.S. Radin. 1963. Microdetermination, isolation and gas-liquid chromatography of 2-hydroxy fatty acids. J. Lipid. Res. 4:130-138.

27. Bentley, R., C.C. Sweeley, M. Makita, and W.W. Wells. Gas chromatography of sugars and other polyhydroxy compounds. Biochem. Biophys. Res. Commun. 11:14-18.

28. Wiig, E.O. W.R. Line, and J.F. Flagg. 1954. Semimicro qualitative analysis, p. 160-164. D. van Nostrand Co., Inc., New York.

29. MacFarlane, M.G., and G.M. Gray. 1957. Composition of cardiolipin. Biochem. J. 67:25P-26P.

30. Olley, J. 1956. Quantitative paper chromatography of lipid constituents, p. 49-55. In G. Popjak, and E. LeBreton. (ed.) Biochemical problems of lipids. Butterworths Scientific Publications, London.

31. Hanahan, D.J. 1960. Lipide chemistry, p. 106-133. John Wiley & Sons, Inc., New York.

32. Silverstein, R.M., and G.C. Bassler. 1964. Spectrometric identification of organic compounds, p. 66-67. John Wiley & Sons, Inc., New York.

33. Akashi, S., and K. Saito. 1960. A branched-saturated C_{15} acid (sarcinic acid) from Sarcina phospholipids and a similar acid from several microbial lipids. J. Biochem. (Tokyo) 47:222-229.

34. Kates, M., G.A. Adams, and S.M. Martin. 1964. Lipids of Serratia marcescens. Can. J. Biochem. 42:461-479.

35. Law, J.H. 1961. Lipids of Escherichia coli, p. 129. Bacteriol. Proc.

36. Kaneshiro, T., and A.G. Marr. 1962. Phospholipids of Azotobacter agilis, Agrobacterium tumefaciens, and Escherichia coli. J. Lipid Res. 3:184-189.

37. Gaby, W.L., H.L. Wolin, and I. Zajac. 1960. The role of phospholipids in the uptake of amino acids by Ehrlich Ascites carcinoma cells. Cancer.Res. 20:1508-1513.

38. Hunter, G.D., and G.N. Godson. 1961. Later stages of protein synthesis and the role of phospholipids in the process. Nature 189:140-141.

39. Kates, M. 1960. Lipolytic enzymes, p. 212-214. In K. Bloch, (ed.) Lipide metabolism. John Wiley & Sons, Inc., New York.

40. Matches, J.R., H.W. Walker, and J.C. Ayres. 1964. Phospholipids in vegetative cells and spores of Bacillus polymyxa. J. Bacteriol. 87:16-23.